Respirometer for Student Use

A simple respirometer for student use can be made with a minimum of equipment and glass construction. The necessary components are shown in fig. 1. The

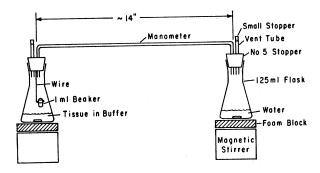


Fig. 1. Diagram of the differential respirometer.

respirometer is of the Fenn type (M. Dixon, 1943: Manometric Methods, 2nd ed.; Macmillan Co., New York), which has a horizontal manometer. Being a differential instrument, employing two similar flasks, there is no need for a thermal barometer. Experiments can be run at room temperature without need of a water bath. The manometer may be constructed from a length of capillary tubing, on which a scale can be marked, or from a discarded Warburg

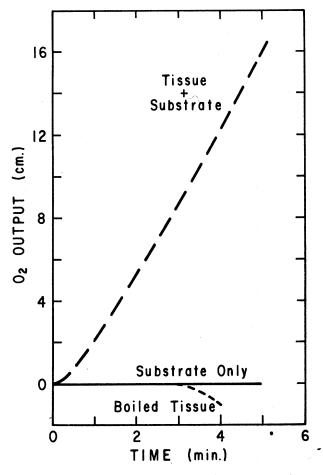


Fig. 2. Demonstration of catalase activity in plant tissue.

manometer. The manometer fluid is merely a droplet of water. The small side-tubes serve as vent tubes and for the introduction of substrates. A shaker mechanism is unnecessary: the apparatus can be handshaken or, more conveniently, magnetic stirrers can be used; in the latter case, foam blocks are placed above each stirrer to eliminate any heat effect from the motors. For those experiments in which ${\rm CO_2}$ is produced, a "KOH cup" in the form of a 1-ml beaker can be suspended on a wire, as shown. It is important to have the manometer capillary in a level position when setting up the apparatus, so that the water droplet does not move due to gravity.

The usual operating procedure is as follows:

- 1. Place a droplet of water in the capillary and move it to a convenient position by use of the slight pressure of a small rubber bulb.
- 2. To the sample flask add the tissue sample or enzyme source (for example, ground-up leaf tissue) suspended in approximately 25 ml of buffer solution. Add an equivalent volume of water to the blank flask.
- 3. With vents open, connect the manometer to both flasks; check for good fit of stoppers and level of manometer.
- 4. Turn on the stirrers and carefully close the vent tubes with small stoppers.
- 5. Observe the droplet and check for background respiration for about 5 minutes; this should be virtually zero. Shut off the stirrers.
- 6. Carefully remove both small stoppers simultaneously and add substrate to the sample flask through the vent tube by means of a drawn capillary dropper or by syringe and needle.
- 7. Close the vent tubes and turn on the stirrers. Observe the movement of the droplet and take readings at appropriate time intervals.
- 8. To prove the presence of enzyme activity, repeat the above experiment with boiled tissue or enzyme; repeat with substrate only.

Typical experimental results, illustrated in fig. 2, are presented for the demonstration of catalase activity in plant tissue, using H_2O_2 as substrate.

It is not necessary to use calibrated glassware and convert readings to actual gas volumes, because raw data appear to be sufficiently illustrative for student purposes.

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